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Session TA2
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Plant and Animal
Gravitational Biology - 1

THE INTERACTION OF MICROGRAVITY AND ETHYLENE ON SOYBEAN GROWTH AND METABOLISM

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INTRODUCTION

Spaceflight has profound effects on plant growth and metabolism, however the initial response of the plants to this unique environment is not known. There are several reports of enhanced ethylene production by plants which have been subjected to earth-based altered-gravity and/or spaceflight conditions. The experiments presented here were designed to determine 1) if spaceflight and/or ground-based microgravity simulations result in increased ethylene production in etiolated soybean seedlings, 2) the physiological impact of gravity-induced enhancement of ethylene production and 3) if removal of atmospheric ethylene ameliorates the observed physiological and metabolic effects of spaceflight.

METHODS

Soybean (*Glycine max* L. [Merr] cv. McCall) seeds were germinated and grown in Biological Research In Canister (BRIC) ground support and flight hardware. The BRIC hardware consists of two light-tight, independent compartments containing 4 passive pressure relief vents which were used for gas sampling ports. Individual surface sterilized seeds (13/compartment) were rolled in germination paper, inserted in a Teflon tube and placed within the BRIC hardware. The seeds were watered and the BRIC hardware was closed and loaded onto the Space Shuttle. For ground control experiments, the hardware was placed either on a clinostat or remained stationary in the vertical position. After the 8-day mission, gas samples were taken and analyzed for ethylene and CO₂ using gas chromatography. Plants were then harvested and analyzed for growth.

These experiments will be continued and expanded upon in the upcoming Collaborative Ukrainian Experiment (CUE), a joint project of the United States and Ukraine focusing on plant science which is scheduled to fly on-board the Space Shuttle (STS-87) in October 1997. In order to remove the atmospheric ethylene from the canisters in-flight without disturbing other canister atmospheric constituents (especially CO₂ and relative humidity), several passive scrubbing techniques were tested and the results are reported below.

RESULTS

Clinorotated soybean seedlings produced nearly twice as much ethylene as the upright stationary controls after 7 days. Space-grown seedlings also produced twice the ethylene as ground-controls. In both the clinostat and spaceflight experiments, there was no difference in the concentration of CO₂.

Root growth was enhanced and shoot growth diminished as a result of clinorotation. Root fresh weight was lower in space-grown plants relative to the ground controls but the root lengths were not different. The shoot/root fresh weight ratio was greater in the space-grown plants whereas it was diminished in the clinorotated plants.

One gram of Purafil[®] pellets in a mesh bag was found to effectively remove ethylene from a half BRIC canister for the duration of the proposed CUE experiment (up to 10 days). Using KMnO₄ (the active ingredient of Purafil[®]) alone did not scrub ethylene consistently and resulted in stunted seedling growth.

CONCLUSIONS

Spaceflight and clinorotation resulted in altered biomass partitioning and increased ethylene production in etiolated soybean seedlings. It is possible that the growth differences were due to the enhanced ethylene concentrations found in space-exposed or clinorotated canisters. We will test this hypothesis during the upcoming CUE experiment.

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STRUCTURE AND G-SENSITIVITY OF ROOT STATOCYTES UNDER DIFFERENT MASS ACCELERATION

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For the development of the conception that the structure and functions of a cell may be modified by the change of the direction of mass acceleration as well as its magnitude, experiments with *Hordeum*, *Lepidium*, and *Lactuca* primary roots on board of the orbital station Mir, biosatellite Bion-10 and Bion-11, on the slow (2 - 4 rpm) and fast (50 rpm) rotating clinostat, and on the centrifuge - clinostat of special construction were carried out. Two main questions were under investigation: (i) the role of gravity in the formulation and maintenance of the polar structure of root statocytes, and (ii) the threshold for detection of g-force by root gravisensors.

Statistical comparison of the spatial localization of main cell organelles (nucleus, amyloplasts, mitochondria) in the statocytes of different plant species grown under microgravity and on the clinostat revealed more similarities than differences. The analysis of the statolith statics and dynamics in experiments carried out with *Lactuca* and *Lepidium* roots exposed to accelerations of 0.005, 0.01, 0.1, and 1 g showed that gravity may be considered as an important though not a single factor that takes part in the functional organization of a statocyte structure. Some additional data on the role of a cytoskeleton in the structural self-organization of the statocyte are presented and considered.

The minimal acceleration acting in the longitudinal direction of a statocyte still capable of influencing the spatial localization of the statoliths was determined to be lower than 0.01 g. When a functional dependence between the lateral stimulation force (g) in the range of 0.005 - 0.1 g and the gravitropic response (R) was expressed as $R = a + b \ln(g)$, the threshold acceleration for gravitropic stimulation of *Lepidium* and *Lactuca* roots were calculated to be 2.6×10^{-3} and 3.7×10^{-3} g respectively. The obtained experimental data are considered from the point of view of the statolith theory.

EXTRACELLULAR PRODUCTION OF TAXANES ON CELL SURFACES IN SIMULATED MICROGRAVITY AND HYPERGRAVITY¹

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INTRODUCTION

Cell suspension cultures of *Taxus cuspidata* produce free and covalently bound taxanes at 1xg on the outer surfaces of cells. This study explores effects of gravitational forces on 'natural' paclitaxel (Taxol™) production and release into the culture medium. Paclitaxel is an antimitotic drug for the treatment of human malignancies viz. ovarian and breast cancer. This research provides a model for the final assembly of extracellular paclitaxel and offers new bioproduct recovery strategies for plant cells in suspension cultures.

METHODS

Cells were grown in 100 ml rotary cell culture bioreactors (Synthecon, Houston TX) for 14 d to simulate microgravity and to produce taxanes on the outer cell surface (1). Taxanes and paclitaxel were localized with monoclonal antibodies (Hawaii Biotechnology Group) to the taxane ring of paclitaxel. Apoptotic cells were distinguished morphologically and histochemically from nonapoptotic cells (2,3). Free taxanes and those bound and released by xylanase were separated on taxil columns (MetaChem Technologies Inc.). Cells were also exposed to 1xg and to 3, and 24xg in a laboratory centrifuge.

RESULTS

Taxanes were localized by monoclonal antibodies on the surfaces of stressed cells. Taxane contents varied according to their physiological state and entry into apoptosis. Taxanes were located together with endonucleases on chromatin of apoptotic nuclei. After solvent extraction of cell suspensions, covalently bound taxanes were detected in cell debris and removed by xylanase treatment. Sites of taxane assembly were associated with cell surface fibrils and with membranes staining for peroxidase containing heavy/transition metals localized by colloidal silver. Cell growth was increased with microgravity to a doubling rate of 7-9 days, but with decreased taxane production. Exposure of cells to Earth's gravity and to hypergravity increased taxane production significantly. Cells continued to grow even at 24xg and survived these forces for over 4 months without subculture. Adaptation was postulated as related to *TCH* (touch) genes for responses to mechanical stress by endoxyloglucanase activity and to calcium-related sensing of gravitational forces (e.g. 4).

CONCLUSION

The final assembly of taxanes including paclitaxel on cell surfaces are responsive to gravitational forces. Sites of surface assembly reveal a confluence of membranes bearing heavy metals and particles that are released into the culture medium either a free or covalent bound taxanes. This work suggests new modifications to current bioproduct recovery strategies (5) using bioreactors suitable for the Space shuttle program.

1. Durzan DJ, Ventimiglia F 1996 US Patent No. 5,5547,866.
2. Havel L, Durzan DJ 1996 Botanica Acta 109: 268-277.
3. Havel L, Durzan DJ 1996 Intl J Plant Sci 157: 8-16.
4. Xu et al. 1995 Plant Cell 7: 1555-1567.
5. Gonda S 1996 MSAD OLMSA NASA HQ and Langley Res Cntr. Advanced Technology Development '96.

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CURRENT PROBLEMS OF SPACE CELL PHYTOBIOLOGY

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The discovery of cell gravisensitivity, including plant cells, focuses increasing attention to an elucidation of the mechanisms involved in microgravity effects at the cellular, subcellular and molecular levels. On the basis of the concept that microgravity has an essential effect on cell metabolism, proliferating and metabolizing cells are the most sensitive to the influence of altered gravity. Microgravity is assumed to induce rearrangements in the cytoplasmic membrane's physical-chemical organization; these changes underlie changes in its permeability (ion transport, receptor functions, bound enzyme activity) and the further chain of sequential changes in cell metabolism. Hence, the main focus in space cell phytobiology to date should be on the investigations of 1) primary events occurring at the membrane level, especially in the cytoplasmic membrane and the tonoplast under the influence of microgravity, 2) messenger systems providing the transduction of primary microgravity effects in the integrated intracellular responses (including Ca, inositol phospholipids, protein kinases, and cyclic mononucleotides), 3) levels of metabolism regulation (gene expression, phytohormones' content and composition, allosteric processes) in altered gravity, 4) photosynthesis and its intensity under the influence of altered gravity, and 5) peculiarities of secondary metabolism in cultured *in vitro* plant cells and organs in microgravity which are the producers of biologically active substances. Possible approaches and objects for carrying out these investigations are discussed.

BIOLOGICAL CONSEQUENCES OF MICROGRAVITY-INDUCED ALTERATIONS IN WATER METABOLISM OF PLANT CELLS

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There is considerable literature, directly or indirectly demonstrating that microgravity environments modify the water metabolism in plant cells. In particular, results obtained in the experiments with tissue cultures have showed: 1) a decrease in the biomass production of flight cultures; 2) a decrease in the relative content of water. So, the relative water content in the flight samples was 10% less for pea tissue culture and 26% less for haplopappus tissue culture than in control samples; 3) decrease the biomass production more than accumulate of dry matter. In contrary to the flight environments, the positive growth reaction in haplopappus tissue culture under clinostating (50 rev/min) was accompanied by an increase in biomass/dry ratio, cell vacuole size and cell volume. In spite of the differences in reaction of the cultured cells to the flight and model environments the obtained data allows to conclude that the change in the biomass/dry matter ratio reflects an alteration growth processes by cell expansion. Consequently, mechanisms by which the plant cells *in vitro* respond to altered gravity conditions involve mechanisms regulating the water exchange. An identification of the mechanisms maintained the water homeostasis of plant cells could clarify the mechanisms underlying adaptation to microgravity.

Expansive growth in plants is one of the most sensitive of plant processes to water deficit. Water plays a vital role in the functioning of diverse cell processes and decrease in its availability in microgravity may be a primary limitation to cell expansion, division and biomass production. Therefore, the above mentioned evidence and theoretical suggestions require evaluation of the gravity effects at this level. The most important aspects related to regulation of cell growth by expansion are discussed.

LOCALIZATION OF CALCIUM IONS IN CHLORELLA CELLS UNDER CLINOROTATION

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Data obtained in the space flight and clinorotation experiments demonstrate numerous rearrangements in the ultrastructural organization of *Chlorella* cells, especially, energetic organelles - mitochondria and chloroplasts. Since the changes in intracellular calcium balance observed in altered gravity was supposed to play an essential role in cell metabolism, we have studied the localization of calcium ions in *Chlorella* cells (strain Larg-1) under clinorotation (3 rev/min) using a pyroantimonate method.

In *Chlorella* cells grown in the stationary conditions various granules of calcium pyroantimonate of different form, size and quantity were observed in different organelles. Thus, some organelles, in particular, a nucleolus, a chloroplast, the mitochondria and the

vacuoles of cell were marked intensively by precipitates, while a diffuse chromatin of nucleus, the cisterns of a dictyosome - more weaker.

The product of cytochemical reaction in mitochondrial matrix and in nucleolus has been observed in a form of small granules. While the sediment of pyroantimonate calcium revealed in a stroma of chloroplasts, around of the starch grain and among thylakoid bundles was observed in a form of more large granules.

Unlike control cells, there was a precipitate of the cytochemical reaction in the hyaloplasm; it was in the form of fine sediment in small quantity. Increased quantity of a precipitate was localized in the mitochondria and a chloroplast and also in the periplasmic space of vegetative cells and around autospores after cytokinesis. The peculiarities of calcium localization in cells and increased volume of a cytochemical reaction product in organelles under clinorotation are assumed to increase a general pool of ionized calcium in cell under the influence of this factor. Obtained data correspond to the hypothesis of regulative role of calcium under impact of external stimulus on cell.

CHANGES OF FATTY ACIDS CONTENT OF PLANT CELL PLASMA MEMBRANES UNDER ALTERED GRAVITY

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Studies were carried out with plasma membranes of pea roots seedling under normal conditions and under 24, 48, 72 and 96 h of clinostatting (2 rev/min). The plasma membranes fraction was isolated by differential centrifugation in stepped density gradient of saccharose. A fatty acid content of lipids was measured by gas-liquid chromatography. Microviscosity of plasma membranes and model phospholipid membranes – liposomes is studied by the method of fluorescent probes.

Lipids of plasma membranes of pea root cells include the following saturated fatty acids: myristic (C_{14:0}), palmitic (C_{16:0}), stearic (C_{18:0}) and unsaturated ones – myristoleic (C_{14:1}), oleic (C_{18:1}), linoleic (C_{18:2}), linolenic (C_{18:3}).

The total content of unsaturated fatty acids increased during clinostatting. There were increases of unsaturated fatty acids mainly at the expense of linoleic and linolenic acid and also a decrease of saturated fatty acids content at the expense of palmitic and stearic acids. Fatty acid composition of the plasma membrane was more variable in composition of plasma membranes was more variable in comparison with phospholipids. Data obtained suggest a high sensitivity of microviscosity indices of liposomes obtained from plasmalemma lipids after 24 h of clinostatting. These data agree with both the changes in a fatty acids content and an increase of the unsaturation index at the final stage (48, 96 h) of clinostatting and may be considered as one of the mechanisms maintaining fluidity of a lipid bilayer of the plasmalemma within certain limits that is homeoviscous membrane adaptation to these conditions.

SIMULATION OF GRAVITY BY NON-SYMMETRICAL VIBRATIONS AND ULTRASOUND

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INTRODUCTION

The primary process of gravity perception involves the displacement of some mass under the gravitational force. In plants gravity perception depends on intracellular displacement of amyloplasts. Gravity perception of plants was commonly studied by clinorotation or centrifugation. However, the resultant forces affect the entire plant. An amyloplast-specific force was based on magnetic ponderomotive forces by high gradient magnetic fields (HGMF), which resulted in the displacement of amyloplasts in statocytes [3] and caused curvature of roots away from and shoots toward the stronger field areas. In addition to HGMF there are other physical principles can induce displacement and/or pressure changes of dense, intracellular particles, including inertial forces due to non-symmetrical vibrations and acoustic forces. The rheology and mechanical properties of plant cells have not been studied thoroughly yet, but available data indicate differences in mechanical properties between amyloplasts and the rest of the cell to be large enough to affect amyloplasts by these forces.

MODEL

If a body such as a columella cell is subjected to oscillations, relatively dense statoliths will move relative to other cellular structures. A non-inertial coordinate system X'O'Y' (Fig. 1), that moves with the cell wall with the velocity V(t) in a stationary system XOY, an inertial force $F_{in}(t) = m a(t)$ (with m = buoyant mass, $a(t)$ = momentary acceleration of the cell) acts on amyloplasts. The viscous friction F_{fr} acts on amyloplasts, that move with the velocity v(t) in respect to the surrounding cytoplasm. Hence, movement of amyloplast can be described by the equation: $m(dv/dt) = \vec{F}_{in} - \vec{F}_{fr}$. If F_{fr} is linearly proportional to v(t) and the mobility in both directions of the oscillation the same, then there would be no net displacement of amyloplasts. But the rheology of the intracellular medium is complex. Thus, non-symmetric oscillations (different accelerations during the positive and negative phase of the oscillation) and the non-linearity of the viscosity of the cytoplasm results in a residual force in the system (so-called vibrational force F_{vb} [1]) and causes displacement of amyloplasts. The displacement depends on the amplitude and the waveform of the oscillations [1,2]. Previous work [2] estimated the residual force with the following assumptions: (a) uniform cytoplasm (no structures); (b) the viscosity η being equal to that of water, if the velocity is less than v_{min} , or $\eta=1.2$ if the velocity is higher than v_{min} , and (c) the net force causes linear amyloplast movement but no rotation. For amyloplast movement the Reynold number $Re = \rho R v / \eta \approx 10^{-6}$ to $10^{-7} \ll 1$ (R - radius of amyloplasts, ρ - density of cytoplasm), and Stoke's approximation can be used [2].

Therefore, the movement of the amyloplast is described by: $m \frac{dv}{dt} = \vec{F}_{in} - \vec{F}_{fr} = ma(t) - 6 \pi \eta R v(t)$, and

$a(t) = \frac{6 \pi \eta R}{m} v(t) + \frac{dv}{dt}$. Solving this equation yields the velocity profile u(t) of the motion of amyloplast vs. cytoplasm.

The vibrational force is estimated as $F_{vb} = \frac{1}{\tau} \int_0^{\tau} F_{fr}[v(t)] dt$ and $S_{\tau} = \int_0^{\tau} v(t) dt$ describes the displacement of

amyloplasts per cycle.

MATERIALS AND METHODS.

2-day old flax (*Linum usitatissimum*) seedlings with straight roots were mounted vertically in a chamber (1×50×50 mm). The chamber was mounted to a vibrator so that the amyloplasts were affected horizontally by the force generated by a vibrator, and vertically by g . The vibrator was controlled by a 12-bit waveform generator (FG-102, Real Time Devices) and amplifier and the produced waveform was measured by an accelerometer. If vibration-induced amyloplast displacement causes curvature, the roots should curve (see Fig. 2). The profile of the tested acceleration is shown in Fig. 3. Estimation of the vibrational force for this profile yield $F_{vb} \approx 0.8 \text{ mg}$, based on the above model and assumptions.

RESULTS AND DISCUSSION

Distribution of the observed root curvatures is shown in Fig. 4. Root curvature depended upon the shape of the waveform, indicating that the roots response was in line with theoretical predictions. If the waveform was reversed, curvature in the opposite direction was obtained. Mean angles for the two directions were $22^{\circ} \pm 6.8$, and $25^{\circ} \pm 6.1$. Since

the tangent of this angle (ϕ) is equivalent to the ratio of horizontal force and the gravity force, the vibration induced force was equivalent to about $0.5g$, which is a rather good compliance of experimental results with the theoretical estimations of $0.8g$, given the crudeness of the model. Future experiments will test various types of oscillations and frequencies. Further development of the theoretical model and optimization of waveforms will be suitable to investigate whether forces exerted by (tethered) amyloplasts or their displacement initiates curvature. Studying the response of columella cells to frequency and waveform of applied oscillations will analyze the rheology of the cytoplasm and the elastic properties of the affected organelles.

ACOUSTIC FORCES

Increasing the vibration frequency leads to the ultrasonic part of the sound spectrum. If ultrasound passes through a mechanically heterogeneous medium, scattering and attenuation transfers a part of the sound momentum to the medium. Consequently, a system of ponderomotive forces affects amyloplasts immersed in the cytoplasm with a different acoustic impedance (density \times velocity of sound), and acoustic flow of the cell interior takes place. Therefore ultrasound will displace amyloplasts inside statocytes. Other organelles have acoustic impedances more similar to cytoplasm and are expected to be affected to a lesser extent. Theoretical estimations demonstrate the validity of the approach. Preliminary experiments with 2 day old flax seedlings with vertical straight roots (submerged in water), which were irradiated by ultrasound (frequency 0.8 MHz, intensity 0.1 W/cm²) have shown root curvature. Future investigations will determine optimal parameters of ultrasound and possible side effects on plants.

CONCLUSIONS

The described studies not only permit the study of rheological characteristics of statocytes and cytoplasm but similar to HGMF may result in novel methods that may substitute the gravity force in a microgravity environment.

REFERENCES

- 1) Blekhman II 1994 *Vibrational mechanics*. Moscow, "Nauka": 198-221
- 2) Kuznetsov OA 1993 *Non-uniform magnetic field and relaxational oscillations as instruments for plant cell exploration*. PhD thesis, Moscow, Russia
- 3) Kuznetsov OA, Hasenstein KH 1996 *Magnetophoretic induction of root curvature*. *Planta* 198: 87-94

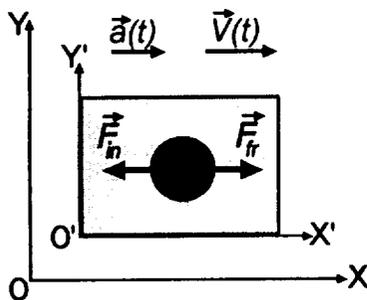


Fig.1 Model of the forces affecting amyloplasts (dark) due to non-symmetrical oscillations of statocytes (shaded).

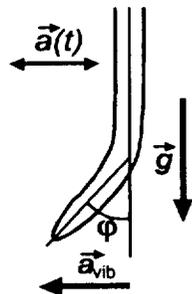


Fig.2 Force ratio of curving root.

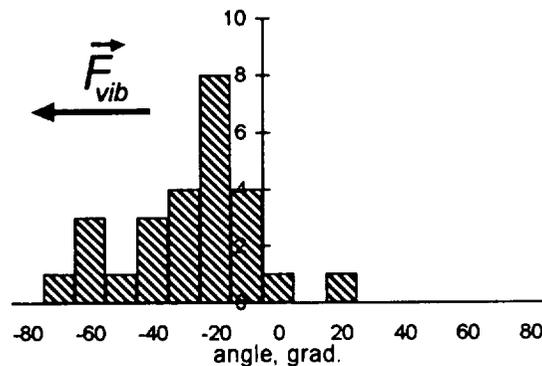
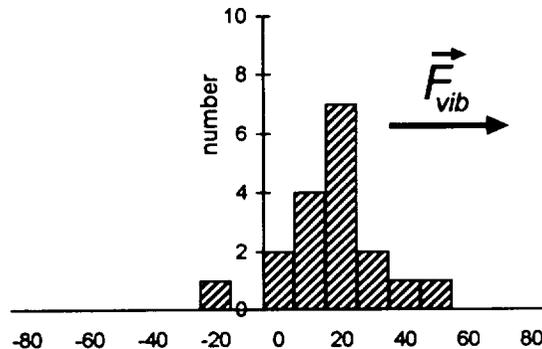


Fig.4 Distribution of curvature of flax roots subjected to the oscillations shown on fig.3.

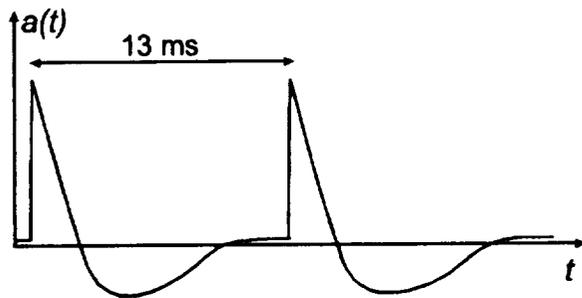


Fig.3 Acceleration profile

RESPONSE TO SIMULATED WEIGHTLESSNESS OF IN VITRO CULTURES OF DIFFERENTIATED EPITHELIAL FOLLICULAR CELLS FROM THYROID

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Aim of this investigation is the study of the molecular modifications occurring in differentiated mammalian cells responding to gravitational changes. Our test system consist of a well characterized clone of differentiated, normal thyroid follicular cells (FRTL5) in long-term culture.

On the MASER-7 sounding rocket (flown by E.S.A. in May, 1996) for the first time we had the opportunity to expose FRTL5 cells to approximately 6 minutes of microgravity, in order to investigate how the gravitational field and the extraterrestrial environment may interfere with hormonal control mechanisms. In this context we evaluated FRTL5 cells responses to thyroid stimulating hormone (TSH) in terms of cAMP production and in terms of cytoskeleton organization and its functional modifications.

In this first experiment we found that in microgravity the TSH effect on cytoskeleton organization and particularly on actin polymerization was impaired to at least 50% as compared to its physiological effect occurring at 1 x g on-ground. cAMP data inexplicably turned out to be very low, dangerously close to background values, but they nevertheless were in the same direction of the above mentioned cytoskeleton experiment.

Due to the serious scarcity of long-term and sounding rocket missions with scientific payloads, particularly of those carrying biological samples and cell cultures, we are now running on-ground experiments which may be important to optimize experimental tools and strategies in preparation to, and in between real flight missions.

Following this approach, we evaluated the FRTL5 cells response to TSH in simulated microgravity obtained by means of a fast-rotating clinostat.

The TSH-dependent signal transduction was evaluated at the following different g-conditions:

- a) simulated weightlessness on a fast-rotating clinostat (60 rpm, approx. equal to 6.48×10^{-3} g);
- b) 1 x g on-ground (control).

Cells pre-equilibrated (for 24 hours) in clinostat, and then acutely stimulated (for 6 min.) with TSH, increase their c-AMP production about 4x with respect to the unstimulated controls. Cells pre-equilibrated (24 hours) and then acutely stimulated (60 min.) with TSH, increase their production of c-AMP about 14x if compared to the unstimulated controls.

In acutely stimulated cells (no adaptation period in clinostat), more subtle differences were found, as compared to their unstimulated controls. Additional experiments are presently under way, to increase the significance of those differences.

In conclusion, our thyroid cultured cells preadapted to clinostat (simulated microgravity conditions) were less responsive to hormonal stimulation in terms of intracellular, more precisely post-receptorial signal transduction, whereas acutely stimulated cells, at the onset of their adaptation to microgravity, are still responding more physiologically to hormonal stimulation. These data may contribute in explaining, on an endocrine basis, a variety of pathophysiological changes repeatedly observed in astronauts, when exposed to long-term space environment. And this is now regarded as an increasingly frequent possibility.